

CLAIMS

What is claimed is:

1. An isolated nucleic acid fragment encoding all or a substantial portion of a plant dihydropicolinate reductase comprising a member selected from the group consisting of:
 - 5 (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2 and 4;
 - (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2 and 4; and
 - (c) an isolated nucleic acid fragment that is complementary to (a) or (b).
- 10 2. The isolated nucleic acid fragment of Claim 1 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:1 and 3.
- 15 3. A chimeric gene comprising the nucleic acid fragment of Claim 1 operably linked to suitable regulatory sequences.
4. A transformed host cell comprising the chimeric gene of Claim 3.
- 20 5. A dihydropicolinate reductase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:2 and 4.
6. An isolated nucleic acid fragment encoding all or a substantial portion of a plant diaminopimelate epimerase comprising a member selected from the group consisting of:
 - 25 (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:7, 9, 11, and 13;
 - (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:7, 9, 11, and 13; and
 - (c) an isolated nucleic acid fragment that is complementary to (a) or (b).
- 30 7. The isolated nucleic acid fragment of Claim 6 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:6, 8, 10, and 12.
8. A chimeric gene comprising the nucleic acid fragment of Claim 6 operably linked to suitable regulatory sequences.
- 35 9. A transformed host cell comprising the chimeric gene of Claim 8.

10. A diaminopimelate epimerase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 7, 9, 11, and 13.

11. An isolated nucleic acid fragment encoding all or a substantial portion of a
5 plant threonine synthase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:16 and 18;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:16 and 18; and
- 10 (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

12. The isolated nucleic acid fragment of Claim 11 wherein the nucleotide
15 sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:15 and 17.

13. A chimeric gene comprising the nucleic acid fragment of Claim 11 operably linked to suitable regulatory sequences.

14. A transformed host cell comprising the chimeric gene of Claim 13.

15. A threonine synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:16 and 18.

16. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine synthase comprising a member selected from the group consisting of:

- 25 (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:20;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:20; and
- 30 (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

17. The isolated nucleic acid fragment of Claim 16 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:19.

18. A chimeric gene comprising the nucleic acid fragment of Claim 16 operably linked to suitable regulatory sequences.

35 19. A transformed host cell comprising the chimeric gene of Claim 18.

20. A threonine synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:20.

21. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine synthase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:22 and 24;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:22 and 24; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

10 22. The isolated nucleic acid fragment of Claim 21 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:21 and 23.

15 23. A chimeric gene comprising the nucleic acid fragment of Claim 21 operably linked to suitable regulatory sequences.

24. A transformed host cell comprising the chimeric gene of Claim 23.

25 25. A threonine synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:22 and 24.

20 26. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine synthase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:26;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:26; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

25 27. The isolated nucleic acid fragment of Claim 26 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:25.

30 28. A chimeric gene comprising the nucleic acid fragment of Claim 26 operably linked to suitable regulatory sequences.

29. A transformed host cell comprising the chimeric gene of Claim 28.

35 30. A threonine synthase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:26.

31. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine deaminase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:29;

- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:29; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

5 32. The isolated nucleic acid fragment of Claim 31 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:28.

33. A chimeric gene comprising the nucleic acid fragment of Claim 31 operably linked to suitable regulatory sequences.

10 34. A transformed host cell comprising the chimeric gene of Claim 33.

35. A threonine deaminase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in SEQ ID NO:29.

36. An isolated nucleic acid fragment encoding all or a substantial portion of a plant threonine deaminase comprising a member selected from the group consisting of:

- 15 (a) an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:31 and 33;
- (b) an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:31 and 33; and
- (c) an isolated nucleic acid fragment that is complementary to (a) or (b).

20 37. The isolated nucleic acid fragment of Claim 36 wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in a member selected from the group consisting of SEQ ID NO:30 and 32.

25 38. A chimeric gene comprising the nucleic acid fragment of Claim 36 operably linked to suitable regulatory sequences.

39. A transformed host cell comprising the chimeric gene of Claim 38.

30 40. A threonine deaminase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO:31 and 33.

41. An isolated nucleic acid fragment encoding all or a substantial portion of a plant S-adenosylmethionine synthetase wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:35.

35 42. A chimeric gene comprising the nucleic acid fragment of Claim 41 operably linked to suitable regulatory sequences.

43. A transformed host cell comprising the chimeric gene of Claim 42.

44. An isolated nucleic acid fragment encoding all or a substantial portion of a plant S-adenosylmethionine synthetase wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:38.

45. A chimeric gene comprising the nucleic acid fragment of Claim 44 operably linked to suitable regulatory sequences.

46. A transformed host cell comprising the chimeric gene of Claim 45.

47. An isolated nucleic acid fragment encoding all or a substantial portion of a plant S-adenosylmethionine synthetase wherein the nucleotide sequence of the fragment comprises all or a portion of the sequence set forth in SEQ ID NO:41.

48. A chimeric gene comprising the nucleic acid fragment of Claim 47 operably linked to suitable regulatory sequences.

49. A transformed host cell comprising the chimeric gene of Claim 48.

50. A method of altering the level of expression of a plant amino acid biosynthetic enzyme in a host cell comprising:

- 15 (a) transforming a host cell with the chimeric gene of Claims 3, 8, 13, 18, 23, 28, 33, 38, 42, 45, and 48; and
- (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of a plant amino acid biosynthetic enzyme in the transformed host cell.

51. A method of obtaining a nucleic acid fragment encoding all or substantially all of the amino acid sequence encoding a plant amino acid biosynthetic enzyme comprising:

- 25 (a) probing a cDNA or genomic library with the nucleic acid fragment of any of Claims 1, 6, 11, 16, 21, 26, 31, 36, 41, 44, and 47;
- (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of any of Claims 1, 6, 11, 16, 21, 26, 31, 36, 41, 44, and 47;
- (c) isolating the DNA clone identified in step (b); and
- (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

30 wherein the sequenced nucleic acid fragment encodes all or substantially all of the amino acid sequence encoding a plant amino acid biosynthetic enzyme.

52. A method of obtaining a nucleic acid fragment encoding a portion of an amino acid sequence encoding a plant amino acid biosynthetic enzyme comprising:

- 35 (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs:1, 3, 6, 8, 10, 12, 15, 17, 19, 21, 23, 25, 28, 30, 32, 35, 38, and 41; and
- (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a portion of an amino acid sequence encoding a plant amino acid biosynthetic enzyme.

53. The product of the method of Claim 51.
54. The product of the method of Claim 52.
5. A method for evaluating at least one compound for its ability to inhibit the activity of a plant biosynthetic enzyme selected from the group consisting of dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase, the method comprising the steps of:
 - 10 (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding a plant biosynthetic enzyme selected from the group consisting of dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosyl-methionine synthetase, operably linked to suitable regulatory sequences;
 - 15 (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the biosynthetic enzyme encoded by the operably linked nucleic acid fragment in the transformed host cell;
 - 20 (c) optionally purifying the biosynthetic enzyme expressed by the transformed host cell;
 - (d) treating the biosynthetic enzyme with a compound to be tested; and
 - (e) comparing the activity of the biosynthetic enzyme that has been treated with a test compound to the activity of an untreated biosynthetic enzyme,
- 25 thereby selecting compounds with potential for inhibitory activity.